REMARKS

Claim Amendments

Claims 1, 3, 14-21, 30, and 33 have been amended and claims 2, 4-13, 22-29, and 31-32 have been previously canceled. New claims 34-36 have been added. Applicant has amended claim 1 so that it is drawn to a chemically modified double stranded nucleic acid molecule having the following features: (1) it comprises a sense strand and a separate antisense strand, each strand having one or more pyrimidine nucleotides and one or more purine nucleotides; (2) each strand of the siRNA nucleic acid molecule is 18 to 27 nucleotides in length; (3) the antisense strand of the nucleic acid molecule comprises 18 to 27 nucleotides that are complementary to a human VEGF RNA comprising SEQ ID NO: 474; (4) the sense strand of the nucleic acid molecule is complementary to the antisense strand, and comprises an 18 to 27 nucloetide portion of the human VEGF RNA sequence; (5) about 50 to 100 percent of the nucleotides in each of the sense and antisense strands of the nucleic acid molecule are chemically modified with modifications independently selected from the group consisting of 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-deoxy, phosphorothioate and deoxyabasic modifications; and (6) one or more of the purine nucleotides present in one or both strands of the nucleic acid molecule are 2'-O-methyl purine nucleotides and one or more of the pyrimidine nucleotides present in one or both strands of the nucleic acid molecule are 2'-deoxy-2'-fluoro pyrimidine nucleotides.

Amended claim 1 is fully supported by the specification as filed, for example, inter alia, at pages 7-9, 10, 11-12, 16-17, 19-25, 32-35, and 41-45.

In addition, claims 14, 15, 18, 19, and 20 have been amended to clarify that 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more of the specified purine or pyrimidine nucleotides has the specified modification. Support for the amendment is found in the specification at, for example, pages 32-35.

In addition, the claims have been further amended merely to correct dependencies and other matters of form.

New claim 34 recites a double stranded nucleic acid wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more of the pyrimidine nucleotides present in the sense strand are 2'-O-methyl pyrimidine nucleotides. Support for new claim 34 can be found in the specification at, for example, pages 32-35.

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New claim 35 depends from claim 19 and recites a method using a double stranded nucleic acid molecule wherein additionally 1, 2, or 3 purine nucleotides in the sense strand are 2'-O-methyl nucleotides. Support for new claim 36 is found in the specification at, for example, pages 32-35.

New claim 36 depends from claim 1 and recites a method of inhibiting the expression of human VEGF RNA comprising administering the nucleic acid molecule of claim 1 to a human subject in need thereof that expresses human VEGF RNA under conditions that allow for inhibition of human VEGF RNA expression. Support for new claim 36 is found in the specification at, for example, pages 13-15 and 50-56.

Amendments to the claims are made without prejudice and do not constitute amendments to overcome any prior art or other statutory rejections and are fully supported by the specification as filed. Additionally, these amendments are not an admission regarding the patentability of subject matter of the canceled or amended claims and should not be so construed. Applicant reserves the right to pursue the subject matter of the previously filed claims in this or in any other appropriate patent application. The amendments add no new matter and applicants respectfully request their entry.

A complete listing of all the claims, in compliance with the revised amendment format, is shown above.

Priority

The Office accords the instant application a priority date of January 26, 2004, which is the filing date of the application. The Office did not accord the instant application the benefit of the earlier priority applications because it alleges that support for the claims is not readily apparent in the priority documents.

Applicant submits that the instant application claims priority, *inter alia*, to PCT/US03/05022, which claims the benefit of provisional application 60/363,124, among other applications. Both PCT/US03/05022 and provisional application 60/363,124 teach the instantly claimed invention. For example, support in PCT/US03/05022 for a chemically modified double stranded nucleic acid comprising a sense strand and antisense strand, wherein the antisense

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strand is complementary to a VEGF nucleotide sequence comprising SEQ ID NO: 474 (GenBank NM_003376) is found in Table I on page 150 and Table II at pages 155-157. Support for the other claim elements in claim 1 can be found throughout the PCT/US03/05022 specification, and particularly at, for example, pages 11, 14, 15, 16, 19, 21, 22-24, 25, 31-33, and 38-42. Support for the other pending claims as amended can be found in PCT/US03/05022 at, for example, pages 15, 17, 18, 21, 23, 25, 31-33, and 49-54. Support in USSN 60/363,124 for a chemically modified double stranded nucleic acid comprising a sense strand and antisense strand, wherein the antisense strand is complementary to a VEGF nucleotide sequence comprising SEQ ID NO: 474 (GenBank NM_003376) is found in Table III, on page 389. Support for the other elements of claim 1 and for the other pending claims as amended can be found in the 60/363,124 application at, for example, pages 9-11, page 12, pages 15-17, 18, 28, and page 40. Thus, the disclosures of the priority documents comply with the requirements of 35 USC 112, first paragraph. Accordingly, Applicant submits that the instant invention is entitled to a priority date of at least March 11, 2002, the filing date of the 60/363,124 application.

The Office observes that the nucleotide sequence of GenBank Accession Number NM_003376 submitted in the IDS filed July 22, 2004 is 1723 nucleotides in length, while the nucleotide sequence of SEQ ID NO: 474 (which is the sequence of GenBank Accession Number NM_003376) is only 649 nucleotides in length. Based on this observation, the Office concludes that GenBank Accession Number NM_003376 and SEQ ID NO: 474 are different sequences with different lengths and contends that priority application 60/363,124 does not support the instant claims directed to a chemically modified double stranded nucleic acid comprising a sense strand and antisense strand, wherein the antisense strand is complementary to a VEGF nucleotide sequence comprising SEQ ID NO: 474 (GenBank NM_003376).

Applicant submits that GenBank Accession Number NM_003376 has different published versions. The version of GenBank Accession Number NM_003376 disclosed in the priority 60/363,124 application, i.e., the version available as of March 11, 2002, the filing date of the 60/363,124 application, has 649 nucleotides. The information for the GenBank Accession

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Number NM_003376 sequence published on November 1, 2000 is represented below. As shown, the sequence available as of November 1, 2000 is 649 nucleotides in length[1].

LOCUS NM_003376 649 bp mRNA linear PRI 01-NOV-2000
DEFINITION Homo sapiens vascular endothelial growth factor (VEGF), mRNA.
ACCESSION NM_003376
VERSION NM_003376.1 GI:4507884
KEYWORDS .
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 649)
AUTHORS Jingjing L, Xue Y, Agarwal N and Roque RS.
TITLE Human Muller cells express VEGF183, a novel spliced variant of vascular endothelial growth factor
JOURNAL Invest. Ophthalmol. Vis. Sci. 40 (3), 752-759 (1999)

The instant application claims priority to and incorporates by reference PCT/US03/05022 in its entirety, which application claims priority to and incorporates by reference 60/363,124 in its entirety. Thus, the instant application properly claims priority to the 60/363,124 application. Both the PCT/US03/05022 and 60/363,124 applications disclose the instant claims, including teaching GenBank Accession Number NM_003376 (SEQ ID NO: 474). Applicant respectfully submits that the instant invention is entitled to a priority date of at least March 11, 2002, the filing date of the 60/363,124 application.

Double Patenting

The provisional rejection of claims 1-33 under the judicially created doctrine of obviousness-type double patenting over claims 1-30 of copending Application No. US Publication Number 20040309832 is maintained.

Applicants submit that Application No. US Publication Number 20040309832 (USSN 10/670,011) has been abandoned. Applicant respectfully requests withdrawal of the obviousness-type double patenting rejection.

[1] The version of GenBank Accession Number NM_003376 having 1723 nucleotides was published on April 29, 2003 and December 22, 2003.

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Withdrawal of Previous Rejections

Applicant gratefully acknowledges the withdrawal of the 35 USC 112, first paragraph, rejection (written description) of claims 1, 3, 14-21, 30 and 33 in view of the amendment to recite SEQ ID NO: 474.

Applicant gratefully acknowledges the withdrawal of the 35 USC 102(a) rejection of claims 1, 3, and 33 as being anticipated by Reich et al. (Molecular Vision, 9: 210-216 (2003)) in view of the amendment to recite that the nucleic acid molecules are chemically modified with 2'-O-methyl or 2'-deoxy-2'-fluoro nucleotides.

Applicant gratefully acknowledges the withdrawal of the 35 USC 103(a) rejection of claims 1, 3, 14-21, 30 and 33 as being obvious over Reich et al. (Molecular Vision, 9: 210-216 (2003)) in view of Parrish (Mol Cell, 6:1077-1087 (2000)), Elbashir (EMBO J, 20: 6877-6888 (20001)), Cook (US 5,587,471), and Schmidt (Nuc Acid Res, 24: 573-581 (1996)) in view of the amendment to recite that the chemically modified nucleic acid molecules comprise a sense strand and antisense strand, wherein the antisense strand is complementary to a VEGF sequence comprising SEQ ID NO: 474.

Objection to the Specification

The Office objects to the amendment filed on June 21, 2006 because it allegedly introduces new matter into the specification. Specifically, the Office alleges that the sequence listing filed with the June 21, 2006 amendment added SEQ ID NO:474, which sequence appears to be new matter. SEQ ID NO: 474 represents GenBank NM_003376, which GenBank number is disclosed in the instant specification. However, the Office contends that SEQ ID NO: 474 in the June 21, 2006 sequence listing has 649 nucleotides and is not the same sequence as the sequence made of record in the IDS filed July 22, 2004 which has 1723 nucleotides.

Applicant submits that the addition of SEQ ID NO: 474 to the sequence listing does not introduce new matter. SEQ ID NO: 474 represents GenBank NM_003376, which GenBank number is disclosed in the instant specification in Table I at page 150 and Table II at pages 155-157. As discussed above, the nucleotide sequence of GenBank NM_003376 that was published at the time of filing the priority applications (i.e., 60/363, 124) has 649 nucleotides. The GenBank NM_003376 submitted in the IDS filed on July 22, 2004 is the sequence published on

April 29, 2003 and December 22, 2003. (Applicant will file a supplemental IDS to submit the GenBank NM_003376 sequence published on November 1, 2000.)

The Office further contends that the addition of SEQ ID NO: 474 in the June 21, 2006 sequence listing adds new matter because GenBank NM_003376 contains thymine residues, where SEQ ID NO:474 has replaced all of the thymine residues with uracil residues. The Office concludes that the replacement of all thymine nucleotides of GenBank NM_003376 with uracil nucleotides in SEQ ID NO:474 represents new matter.

The Applicants respectfully submit that SEQ ID NO:474 is not new matter for the reasons set forth herein below. Accordingly, Applicants should not be required to cancel the addition of SEQ ID NO:474 to the sequence listing.

35 USC §112, Second Paragraph, Rejection

Claim 16 has been rejected under 35 USC §112, second paragraph, as being indefinite because it recites "wherein the fragment" and there is insufficient antecedent basis in claim 1, from which claim 37 depends, for the term "fragment".

Applicant submits that claim 16 has been amended to remove reference to a "fragment comprising" said sense strand, thereby rendering the rejection moot. Applicant respectfully requests the withdrawal of 35 USC §112, second paragraph, rejection.

35 USC §112, First Paragraph, Rejections

Written Description/New Matter

Claims 1, 3, 14-21, 30 and 33 have been rejected under 35 USC § 112, first paragraph, as allegedly failing to comply with the written description requirement. Applicants respectfully traverse the rejection.

The Office states that SEQ ID NO:474 corresponds to GenBank number NM_003376 as disclosed in Tables I and II of the instant application at pages 150-157. However, the Office asserts that the addition of SEQ ID NO: 474 adds new matter because SEQ ID NO: 474 is not the same sequence as GenBank number NM_003376 because it has 649 nucleotides while GenBank number NM_003376 has 1723 nucleotides and because SEQ ID NO:474 has all of the thymine nucleotides of GenBank NM_003376 replaced with uracil nucleotides.

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As explained above, the nucleotide sequence of GenBank number NM_003376 published at the time of filing the priority applications (November 1, 2000) has 649 nucleotides and is the same sequence as SEQ ID NO: 474. Thus, SEQ ID NO: 474 and GenBank number NM_003376 are the same sequence.

Furthermore, Applicants respectfully disagree that the replacement of thymine residues with uracil residues in SEQ ID NO:474 adds new matter. The application teaches throughout the specification that the siRNA molecules are complementary to and target VEGF RNA sequences. For example, the specification teaches that:

> In one embodiment, the invention features a siNA molecule having RNAi activity against VEGF and/or VEGFr **RNA** wherein the siNA molecule comprises sequence complementary to any **RNA** having VEGF and/or VEGFr encoding sequence, such as those sequence having GenBank accession Nos shown in Table I." (emphasis added) Specification, page 8.

Table I includes GenBank Accession No. NM_003376. Thus, the specification clearly contemplates siRNA molecules comprising nucleotides that are complementary to human VEGF **RNA** nucleotide sequence comprising SEQ ID NO:474.

Furthermore, a review of the GenBank database shows the following information for NM_003376:

> LOCUS NM_003376 649 bp mRNA linear PRI 01-NOV-2000
> DEFINITION Homo sapiens vascular endothelial growth factor (VEGF), mRNA.
> ACCESSION NM_003376
> VERSION NM_003376.1 GI:4507884
> KEYWORDS .
> SOURCE Homo sapiens (human)
> ORGANISM Homo sapiens
> Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
> Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.

Applicants note that NM_003376 is identified as an "mRNA" sequence having 649 nucleotides. Accordingly, one of ordinary skill in the art would have known that the RNA sequence is the same as the reported sequence, except with uracils substituted for thymines. Such substitution would have easily been within the knowledge and skill of the ordinary skilled artisan.

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One of skill in the art would have understood that the applicants were in possession of the invention at the time the application was filed. Therefore, the claims have adequate written description. Applicants respectfully request withdrawal of the rejection.

35 USC §103 Rejections

Claims 1, 3, 14-21, 30 and 33 have been rejected under 35 USC §103 as being obvious over GenBank Accession Number NM_003376 in view of Reich et al. (Mol Vision, 9:210-216 (2003)), Elbashir (EMBO J, 20:6877-6888 (2001)), Matulic-Ademic (US Patent No. 5,998,203), and Parrish et al (Molecular Cell, Vol. 6, 1077-1087, 2000). Applicant respectfully traverses the rejection.

The Office relies on GenBank Accession Number NM_003376 for its teaching of the sequence of VEGF comprising SEQ ID NO: 474, but acknowledges that GenBank Accession Number NM_003376 does not teach an siRNA molecule that is complementary to VEGF. The Office relies on Reich for teaching siRNA inhibitors of human VEGF expression consisting of a sense and antisense strand targeted to human VEGF. Reich does not teach chemical modification of siRNA. The Office relies on Elbashir for its teaching of siRNA having 21-23 nucleotides and alleges that Elbashir teaches modification of the internal nucleotides with 2'-deoxy or 2'-O-methyl modifications. The Office relies on Matulic-Ademic to teach chemical modifications of ribozyme molecules, including inverted abasic moieties, 2'-O-methyl, 2'-deoxy-2'fluoro, and 3'-phosphorothioate modifications. The Office relies on Parrish for allegedly teaching chemically synthesized double stranded siRNA molecules comprising various modifications in the sense and antisense strand, including 2'-deoxy-2'-fluoro modifications. The Office recognizes that Parrish teaches that certain modifications are not tolerated on the antisense strand.

The Office argues that it would have been obvious to make a chemically modified siRNA molecule comprising a sense strand and antisense strand, wherein the antisense strand is complementary to a VEGF nucleotide sequence comprising SEQ ID NO: 474 using the sequence taught by GenBank Accession Number NM_003376, the motivation of Reich et al. and following the methods of Elbashir et al., Matulic-Ademic et al., and Parrish et al. Specifically, the Office argues that it would have been obvious to incorporate at least one 2'-O-methyl or 2'-

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deoxy-2'-fluoro modification into a chemically synthesized siRNA molecule complementary to a VEGF sequence comprising SEQ ID NO: 474 since Elbashir et al, Matulic-Ademic, and Parrish et al. taught that various modifications have been incorporated into double stranded nucleic acids to facilitate uptake of the nucleotide and since these modifications were known in the art to add benefits to double stranded nucleic acids, such as protection from exonuclease degradation, as taught by Elbashir et al., Matulic-Ademic et al., and Parrish et al.

The Office further argues that Elbashir et al., Matulic-Ademic et al., and Parrish et al. demonstrate that it was well known in the art to incorporate two or more modifications, including 2'-O-methyl and 2'-deoxy-2'-fluoro nucleotide modifications, into oligonucleotides and also demonstrate the routine nature of testing various chemical modifications for optimization and stabilization of a double stranded duplex. The Office concludes that one would have been motivated to test modifications that were known to benefit oligonucleotide delivery.

The Office finally argues that one would have a reasonable expectation of success to apply each of the claimed modifications to the siRNA molecules taught by Reich because the chemistry was well known in the art at the time the invention was made, as evidenced by Elbashir et al., Matulic-Ademic et al., and Parrish et al., and one would reasonably expect such modifications to benefit siRNA as well.

Under 35 U.S.C. § 103(a), to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the references, when combined must teach or suggest all the claim limitations. *See* MPEP §2143.

None of these references, alone or in combination, make obvious the presently claimed double stranded nucleic acid constructs because the cited references do not teach or suggest all of the claim elements. None of the references teach or suggest a siRNA molecule in which about 50 to 100 percent of the nucleotide positions in one or both strands of the nucleic acid molecule are chemically modified with 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-deoxy, phosphorothioate and/or deoxyabasic modifications. Furthermore, none of the references teach or suggest a siRNA molecule in which one or more of the purine nucleotides present in one or both strands

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are 2'-O-methyl purine nucleotides and one or more of the pyrimidine nucleotides present in one or both strands are 2'-deoxy-2'-fluoro pyrimidine nucleotides.

As stated by the Office, neither GenBank Accession Number NM_003376, nor Reich et al teach or suggest a chemically modified siRNA molecule. Matulic-Ademic, which is directed to ribozyme technology, also fails to teach or suggest a chemically modified siRNA molecule. Elbashir is the only cited reference that mentions chemical modification of short interfering RNA. However, not only does Elabshir fails to teach selective incorporation of 2'-O-methyl guanine and 2'-O-methyl adenine residues into siRNA, but it expressly teaches away from the use of highly modified siRNA constructs, such as 2'-deoxy and 2'-O-methyl modified constructs. Specifically, Elbashir teaches that extensive substitution with 2'-deoxy or 2'-O-methyl modifications abolishes RNAi (see Figure 4 and pages 6881-6882). Further, given that Elbashir fails to mention 2'-deoxy-2'-fluoro modification, it certainly fails to teach 2'-deoxy-2'-fluoro pyrimidine nucleotides.

The Office also relies on Parrish; however, Parrish does not teach chemically synthesized double stranded siRNA molecules comprising 2'-deoxy-2'-fluoro modifications, much less 2'-deoxy-2'-fluoro pyrimidine modifications. Instead, Parrish teaches long dsRNA molecules, not chemically synthesized short interfering RNA molecules, as the Office alleges. All of the 2'-deoxy-2'-fluoro modifications taught by Parrish were introduced by enzymatic incorporation of 2'-deoxy-2'-fluoro uridine nucleotides into long double stranded RNA molecules (>700 nt), **not** selective synthetic incorporation of 2'-deoxy-2'-fluoro uridine and 2'-deoxy-2'-fluoro cytidine nucleotides (collectively 2'-deoxy-2'-fluro pyrimidine nucleotides) into short interfering RNA molecules. Furthermore, Parrish fails to teach or suggest a siRNA molecule wherein about 50 to 100% of one or both strands are chemically modified and wherein one or more of the purine nucleotides present in one or both strands of the nucleic acid molecule are 2'-O-methyl purine nucleotides and one or more of the pyrimidine nucleotides present in one or both strands of the nucleic acid molecule are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In fact, Parrish expressly teaches that modifications of the antisense strand decrease RNAi activity (see pages 1081 and 1082, Figures 5 and 6). Thus, Parrish expressly teaches away from highly modified siRNA constructs, such as the instantly claimed constructs.

Thus, none of the cited references, alone or in combination, teach the specific pattern of chemical modification recited in the claims; that is, 2'-O-methyl purine nucleotides in one or

both strands and 2'-deoxy-2'-fluoro pyrimidine nucleotides in one or both strands of the nucleic acid molecule, where about 50-100% of each strand are chemically modified with 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-deoxy, phosphorothioate and/or deoxyabasic modifications. Furthermore, a double stranded nucleic acid molecule having a specific pattern of chemical modification is not a structure that would be rendered obvious by a general knowledge in the art of chemical modification of other oligonucleotides, such as ribozymes and antisense.

Despite the lack of teaching in the cited references, the Office suggests that it would have been obvious to make double stranded nucleic acid molecules having chemical modifications with a reasonable expectation of success because chemical modification of oligonucleotides adding stability and specificity to the oligonucleotides had been shown to benefit antisense and ribozymes and thus were known in the art and would be expected to benefit siRNA as well. (Office Action, page 17).

However, Applicant submits that antisense and ribozyme art, such as the disclosure of Matulic-Ademic, is not analogous art to siRNA technology and should not be the basis for an obviousness rejection. Any reference or general knowledge cited to demonstrate obviousness must be analogous art. The reference must either be in the field of applicant's endeavor or, if not, then be reasonably pertinent to the particular problem with which the inventor was concerned." *In re Oetiker*, 977 F.2d 1443, 1447 (Fed. Cir. 1992).

Antisense and ribozyme art is not reasonably pertinent to chemically modified siRNA molecules that target VEGF RNA. Antisense molecules are substantially single-stranded prior to interacting with their target, while siRNA is almost completely in a duplex form; it is well known to those skilled in the art that single-stranded nucleic acid is more susceptible to nuclease attack than is double-stranded nucleic acid. Antisense molecules will tolerate substantial 5' and 3' terminal modifications; in contrast the activities of siRNAs are almost completely destroyed by attaching modifications to the 5' end of the antisense strand of the siRNA. The activity of an antisense molecule is destroyed by modifications that alter the DNA-like structure at the core of molecule. It was not clear in 2001 whether the siRNA duplex would need to maintain an RNA-like structure or whether other structures would be permitted.

Likewise, ribozymes are non-analogous art to siRNA. Ribozymes are substantially single-stranded prior to interacting with their target, while siRNA is almost completely in duplex form; it is well known to those skilled in the art that single-stranded nucleic acid is more

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susceptible to nuclease attack than is double-stranded nucleic acid. Additionally, ribozymes will tolerate substantial 5' and 3' terminal modifications. In contrast, the activity of siRNA molecules is almost completely destroyed by attaching modifications to the 5' end of the antisense strand of the siRNA. Also, unlike siRNA molecules, ribozymes must form a complex RNA secondary structure to be active.

At the priority date of the present application, those of ordinary skill in the art understood that there were different structural features of nucleic acids required for activity in each of ribozyme and siRNA technologies because the mechanism of action of these nucleic acids differed in each. Significantly, the mechanism of siRNA had not yet been explored to the extent that one of ordinary skill in the art understood or could predict the effect of various types and positions of chemical modifications on the activity of a double stranded nucleic acid molecule.

In fact, contrary to the Office's contention, none of the art at the time of filing provides any insight into whether highly modified double-stranded siRNA nucleic acid constructs, such as the claimed siRNA constructs, would function. Indeed, Elbashir expressly teach away from highly modified siRNA constructs. Elbashir teaches that extensive substitution with 2'-deoxy or 2'-O-methyl modifications abolishes RNAi activity. In a section tellingly entitled, "The siRNA User Guide," Elbashir expressly teaches away from highly modified siRNA constructs:

"The siRNA User Guide"

> Efficiently silencing siRNA duplexes are composed of 21 nt sense and 21 nt antisense siRNAs and **must** be selected to form a 19 bp double helix with 2 nt 3'-overhanging ends. 2'-deoxy substitutions of the 2 nt 2'-overhanging ribonucleotides do not affect RNAi, but help to reduce the costs of RNA synthesis and may enhance RNAse resistance of siRNA duplexes. **More extensive 2'-deoxy or 2'-O-methyl modifications reduce the ability of siRNAs to mediate RNAi**, probably by interfering with protein association for siRNAp assembly. (emphasis added).

(see, Elbashir et al., page 6885; see also Tuschl et al., U.S. Publ. No. 2002/0086356, paragraphs [0178] to [0179]).

Because the only teaching in the cited art addressing the issue of the degree of modifications tolerated in siRNA molecules expressly states that more than a few end modifications should be avoided, it could not have been obvious to make the highly modified constructs now being claimed with a reasonable expectation of success.

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Furthermore, in the time period of about 2000-2001 the high potency of siRNAs (as compared to antisense and ribozymes) tended to suggest that no additional chemical modification of the molecules would be necessary. It was common knowledge to those skilled in the art at the time of the invention that single stranded RNA and DNA is much more susceptible to nuclease attack than double stranded nucleic acids. Thus, it was thought the relatively unstructured antisense and ribozyme nucleic acid molecules would be expected to require additional stabilization while the substantially double-stranded siRNA molecules would not. An example of this thinking is seen in Elbashir I (EMBO Journal, 20:6877-6888 (2001)) and Tuschl (U.S. Publ. No. 2002/0086356), where an emphasis was placed on modifying the 3' single stranded ends of the siRNA, with little effort made to modify the double stranded 5' ends. *See,* p. 6881, "*2'-deoxy- and 2'-O-methyl-modified siRNA duplexes*;" p. 6884, "*Sequence effects and 2'-deoxy substitutions in the 3' overhang.*"

The methods paper of Elbashir II (Methods 26:199-213 (2002)) best exemplifies the mindset of the day, that additional chemical modifications are unnecessary for effective RNAi activity. This paper gives specific instructions for designing and carrying out an RNAi experiment. On page 202, Protocol 1 (step 2) states that:

> Independent of the selection procedure described in Fig. 2, synthesize the sense siRNA as 50-(N19)TT, and the sequence of the antisense siRNA as 50-(N'19)TT, where N'19 denotes the reverse complement sequence of N19. N19 and N'19 indicate ribonucleotides; T indicates 2'-deoxythymidine.

Thus, RNA duplexes with dTdT 3' ends were considered the correct substrate for carrying out RNAi experiments. The terminal TT was there primarily to make chemical synthesis easier and less expensive, although some minor protection from single-stranded ribonucleases was also considered a possibility (Elbashir I, Elbashir II). Finally, Elbashir II makes specific mention of four suppliers of siRNA duplexes for RNAi research; all four companies supply the reagents in the standard form described in Protocol 1 of Elbashir II.

As stated above, there was no motivation to seek chemically modified siRNAs during the period in question. Elbashir I is the only paper from the period that describes a significant attempt to modify siRNAs away from their own standard of RNA with TT overhanging ends.

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In the section entitled "*2'-deoxy- and 2'-O-methyl-modified siRNA duplexes*" (see pages 6881-6882), Elbashir describes the effect of chemical modification on the activity of the siRNA duplex to mediate RNAi. The authors state:

> To assess the importance of the siRNA ribose residues for RNAi, duplexes with 21 nt siRNAs and 2 nt 3'-overhangs with 2'-deoxy- or 2'-O-methyl-modified strands were examined (Figure 4). Substitution of the 2 nt 3'-overhangs by 2'-deoxynucleotides had no effect and even the replacement of two additional ribonucleotides by 2'-deoxynucleotides adjacent to the overhangs in the paired region produced significantly active siRNAs. Thus, 8 out of 42 nt of a siRNA duplex were replaced by DNA residues without loss of activity.

Thus, while 2'-deoxy substitutions at the 3'-terminal positions were permitted, there was no mention of any active siRNAs using 2'-O-methyl modifications, even at the terminal positions. Furthermore, because complete substitution of one or both siRNA strands with either 2'-deoxy or 2'-O-methyl residues resulted in a complete loss of RNAi activity, (as demonstrated in both Tuschl and Elbashir I) the results of Elbashir I suggests that modification of internal nucleotides positions reduced the ability of siRNAs to mediate RNAi, probably by interfering with protein interactions or siRNP assembly.

It was not until much later in 2003 that reports began appearing in the scientific literature regarding the use of chemical modifications other than 3'-terminal 2'-deoxy substitutions in siRNAs. *See, e.g.,* Chiu and Rana, 2003, RNA, 9:1034-1048; Allerson *et al.,* 2005, *J. Med. Chem.* 48, 901. It is readily apparent from the publication record that those working in the RNAi field initially followed the teachings of Elbashir, outlined above, in designing siRNAs for experimental work. Only more recently has the use of chemical modifications become generally accepted.

Therefore, no motivation existed at the time of the invention to cleave VEGF RNA using siRNA molecules chemically modified with 2'-O-methyl purine and 2'-fluoro pyrimidine modifications. In fact, because the *only* teaching in the cited art addressing the issue of the degree of modifications tolerated in siRNA molecules expressly states that more than a few end modifications should be avoided, it could not have been obvious to make the highly modified constructs now being claimed with a reasonable expectation of success. The present claims go directly against the express teachings of the art. Thus, due to the teachings of Elbashir (and Tuschl), there was no reasonable expectation of success in using extensively chemically modified siRNA molecules with, *e.g.,* 2'-O-methyl and 2'-fluoro modifications.

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For the reasons discussed above, the cited references, alone or in combination, do not render the instantly claimed chemically modified double stranded nucoeic acid molecules obvious. Accordingly, Applicant respectfully requests withdrawal of the 35 USC §103 Rejection.

Conclusion

In view of the foregoing amendments and remarks, the applicant submits that the claims are in condition for allowance, which is respectfully solicited. If the examiner believes a teleconference will advance prosecution, she is encouraged to contact the undersigned as indicated below.

Respectfully submitted,

Dated: May 1, 2007

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